

Supplemental Figures

Learning & Memory

Reversal learning in *Drosophila* larvae

Nino Mancini^{1*}, Sia Hranova², Julia Weber¹, Aliće Weiglein¹, Michael Schleyer¹, Denise Weber², Andreas S. Thum², Bertram Gerber^{1,3,4*}

¹Department of Genetics, Leibniz Institute for Neurobiology (LIN), 39118 Magdeburg, Germany; ²Institute for Biology, University of Leipzig, 04103 Leipzig, Germany; ³Institute for Biology, Otto von Guericke University, 39106 Magdeburg, Germany; ⁴Center for Behavioral Brain Sciences (CBBS), 39106 Magdeburg, Germany.

*Correspondence to:

Nino.Mancini@lin-magdeburg.de

Bertram.Gerber@lin-magdeburg.de

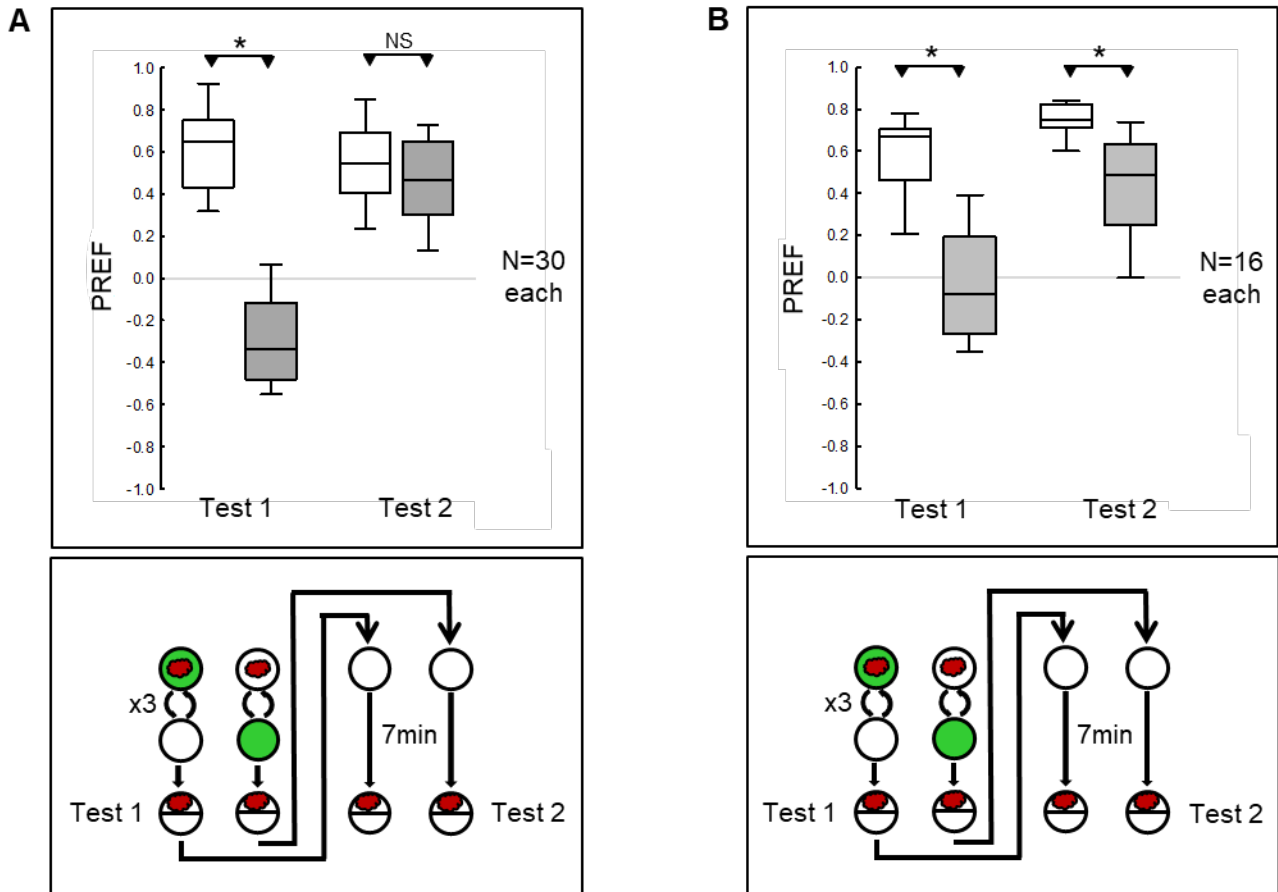


Figure S1: Temporal stability of memory, revisited

Following up on the experiment displayed in Figure 2C, this experiment sought to ascertain whether associative memory remains detectable after an initial test and a 7 min waiting period. **(A)** In a repetition of the experiment displayed in Figure 2C, the larvae were trained by either paired or unpaired presentations of odour (red cloud) and reward (green fill of circle indicating a Petri dish with fructose and, as substrate, agarose) and tested for their odour preference (Test 1); then, the larvae were transferred to a Petri dish with only the agarose substrate (white fill of circle) for a 7 min waiting period and tested again for their odour preference (Test 2). In the first test, the larvae behaved according to the preceding training phase. In this dataset, this effect had vanished by the time of the second test. **(B)** Repetition of the experiment in (A). In this case, too, the larvae behaved according to the preceding training in the first test. In this dataset, this memory was retained until the second test 7 min later. Data are displayed as box plots, the middle line showing the median, the box boundaries the 25 and 75 % quantiles, and the whiskers the 10 and 90 % quantiles. Sample sizes are given within the figure. * and NS refer to MWU comparisons between groups (* $P < 0.05$ corrected according to Bonferroni-Holm and NS $P > 0.05$).

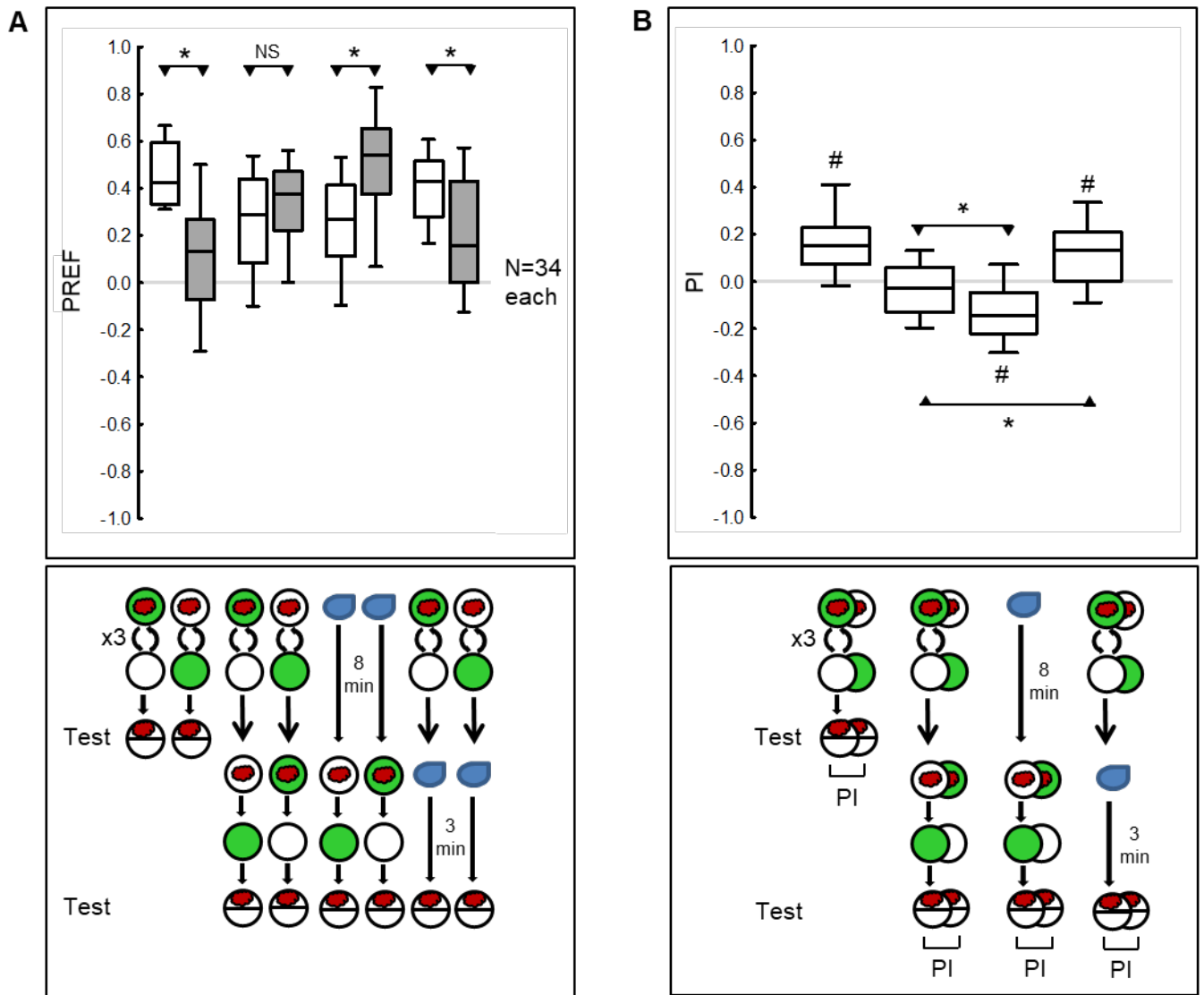


Figure S2: Shortened version of the absolute appetitive reversal learning paradigm

The larvae were trained and tested as in Figure 5, except that the duration of individual training trials was 1 min each. **(A)** The larvae were tested for their odour preference either (i) immediately after a one-phase training, (ii) after training with reversed contingencies in the first and the second training phase, (iii) after omitting the first training phase, or (iv) after omitting the second training phase. **(B)** Performance indices calculated from the preference scores in (A). Positive and negative PIs indicate appetitive memory related to the first and the second training phase, respectively. The performance indices after reversed-contingency training were less negative than when the first training phase was omitted, suggesting a persisting impact from the first training phase. In turn, after reversed-contingency training the performance indices were more negative than when the second training phase was omitted, suggesting an impact from the second training phase. Sample sizes are given within the figure. * and NS refer to MWU comparisons between groups, # refers to OSS comparisons to chance levels i.e. to zero (*, # $P < 0.05$ corrected according to Bonferroni-Holm and NS $P > 0.05$). Other details as in Figure S1.

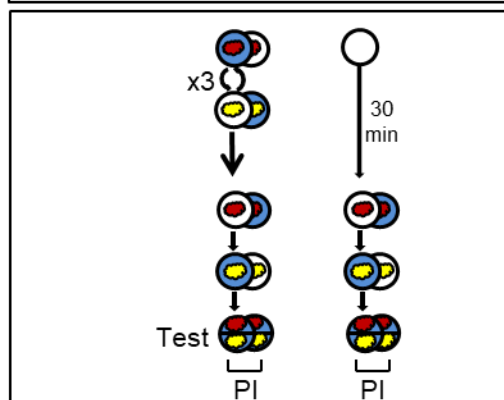
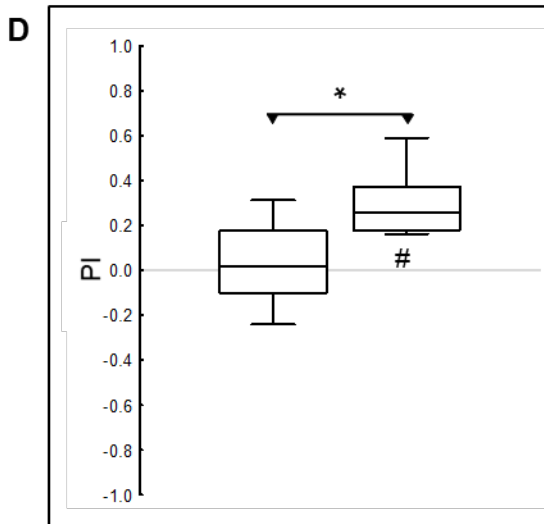
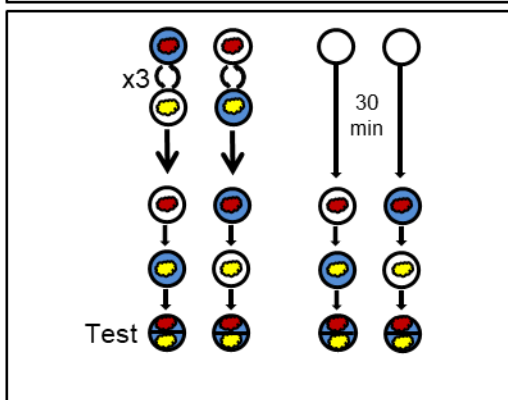
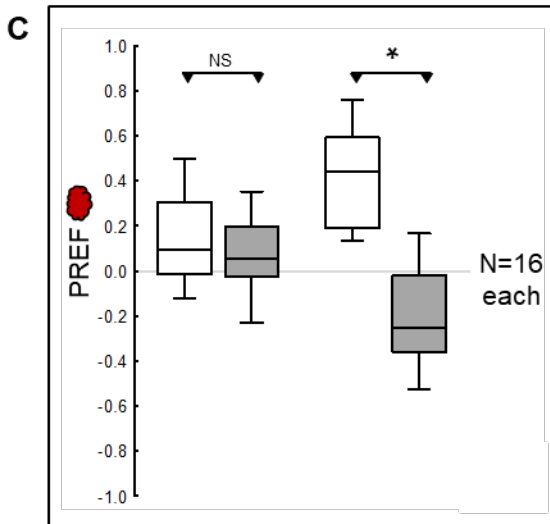
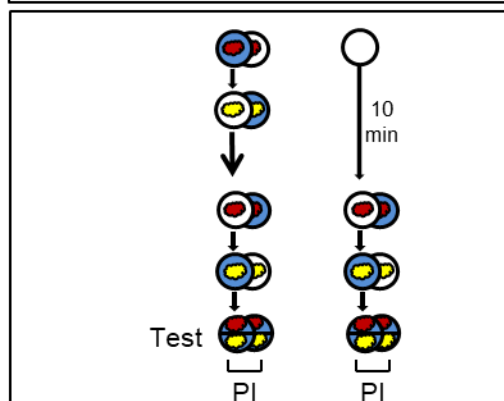
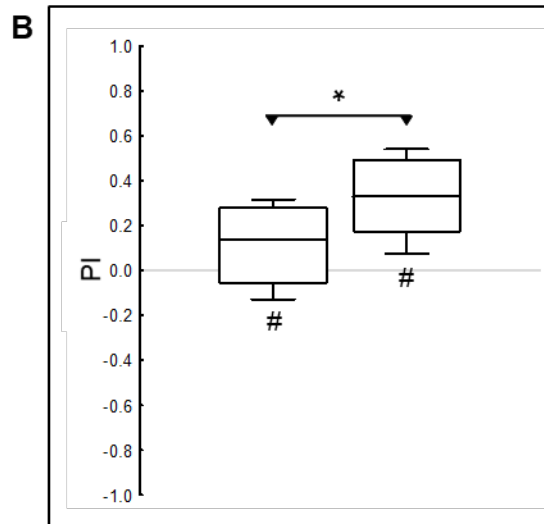
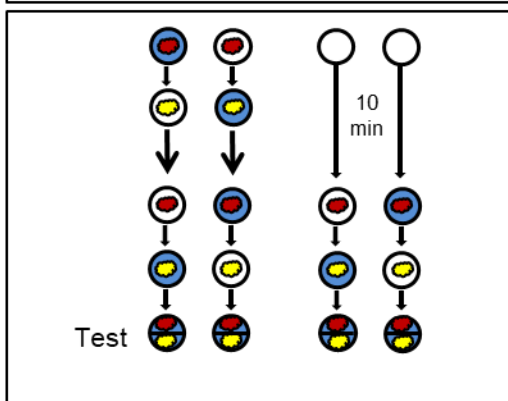
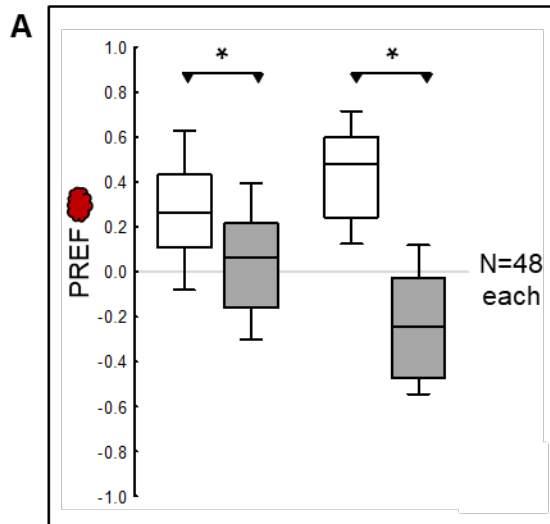


Figure S3: *Increasing the amount of cycles in the first training phase makes differential aversive reversal learning more difficult*

(A) Repetition of two experimental conditions from Figure 8, Figure 9. The larvae were tested after training with reversed contingencies in the first and the second training phase, or after omitting the first training phase. Preference scores (PREF) reflect preference for n-amyl acetate (red cloud). **(B)** Performance indices calculated from the preference scores in (A). Positive PIs indicate aversive memory related to the second training phase. The performance indices after reversed-contingency training were less positive than when the first training phase was omitted, suggesting a persisting impact from the first training phase. In addition, the performance indices after reversed-contingency training were significantly positive, suggesting behaviour in accordance with the second training phase. **(C)** As in (A), except that three cycles were given in the first training phase instead of one cycle. **(D)** Performance indices calculated from the preference scores in (C). As in (B), a persisting impact from the first training phase was detectable, since the performance indices after reversed-contingency training were less positive than when the first training phase was omitted. As expected, having more training cycles in the first training phase makes reversal learning more difficult. Indeed, with three training cycles in the first training phase, the impact of the second training phase was undetectable. Sample sizes are given within the figure. * and NS refer to MWU comparisons between groups, # refers to OSS comparisons to chance levels i.e. to zero (* $P < 0.05$ corrected according to Bonferroni-Holm, # $P < 0.05$ and NS $P > 0.05$). Other details as in Figure S1-2.